Legionella update

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Huit cas de légionellose signalés à Montpellier dont un mortel

Legionnaires' Disease Sickens 127
Largest outbreak ever in New York City kills 12 in South Bronx

Légionellose à New York: le pic est passé

Légionellose : le stade Jean Biart frappé d'interdiction
July 21st 1976 - Philadelphia

- 58th Convention of the American Legion at the Bellevue-Stratford Hotel
- > 4000 World War II Veterans with families & friends
- 600 persons staying at the hotel
- July 23rd: convention closed
- Explosive epidemic of febrile illness with pneumonia

⇒ Searching for the causative agent? (David Fraser: CDC – Atlanta)
  - Influenza virus?
  - Heavy metal intoxication?
  - Toxin?
  - 2603 toxicology tests
  - 5120 microscopy exams
  - 990 serological tests
July 22nd – August 2nd

**Clinical picture**
- High fever
- Coughing
- Breathing difficulties
- Chest pains

Exposed Population = people staying in the lobby or outside the Bellevue Stratford Hotel <<Broad Street Pneumonia>>
- 221 persons were infected (182+39 <<Broad Street Pneumonia>>)
- 34 patients died (29+5)
Outbreak, Philadelphia, 1976

Total number = 221

Number of cases

July
Convention of the American Legion
August

Convention
Autre
December 1976 – January 1977

Joseph McDade – discovery of new bacteria genus

⇒ *Legionella pneumophila*

Development of diagnostic tests including Serological assays and culture

95% of infected patients had positive titer for *L. pneumophila*
Looking back

Previously unresolved outbreaks

- 1968 in Pontiac, Michigan: 95% of the persons working in the same building, 144 cases
- 1965 in Washington: outbreak with 81 cases including 14 death in a hospital
- 1957 in Austin, Minnesota: 78 cases
- 1942 Fort Bragg, North Carolina: 40 soldiers
1st Outbreaks in Europe

Spain 1973 and 1980
In 2015, ≈ 58 distinct species

Legionella dumoffii

95% of infections caused by *L. pneumophila* serogroup 1

except *L. longbeachae* frequent in Australia and New-Zealand
Microbiological characteristics

- Aerobic Gram negative coccobacilli
  - Mobile, non-sporulating, noncapsulated, facultative intracellular parasite
  - LPS O antigens \( \Rightarrow \) serogroups more than 60s
- Catalase +, Oxydase variable
- Inert
  - No fermentation of carbohydrates, no nitrate reduction, no urease
- Nutritionally fastidious
  - In vitro nutritional requirement for growth
  - Auxophotrophism
  - Aminoacids as C-source: L-cystein and other aa
  - Iron salt

Specific media for culture
Environnements
Associated with complex biofilm communities

- Adapted for survival and replication within protozoa
  - Including amoeba and ciliates
- Nutrient supply
  - Including amino acids
- Protection against
  - Temperature alteration, flow effects and chemicals
Epidemiology

• Both sporadic and epidemic form

| Table 6. Ten largest reported clusters of Legionnaires’ disease, 2008–2013 |
|---|---|---|---|---|
| Rank | Reporting country | Year of reporting | Number of cases | Probable setting of infection | Probable source |
| 1 | Spain | 2010 | 51 | Community-acquired | Cooling tower |
| 2 | Spain | 2012 | 39 | Community-acquired | Decorative fountain |
| 3 | Portugal | 2012 | 36 | Community-acquired | Unknown |
| 4 | Spain | 2009 | 25 | Community-acquired | Unknown |
| 5 | United Kingdom | 2012 | 23 | Community-acquired | Spa pool |
| 6 | Spain | 2010 | 22 | Community-acquired | Water system |
| 7 | Poland | 2010 | 19 | Community-acquired | Water system |
| 8 | Spain | 2012 | 18 | Travel-associated | Pool |
| 9 | United Kingdom | 2010 | 15 | Community-acquired | Multiple unknown sources |
| 10 | Spain | 2008 | 14 | Community-acquired | Unknown |

• Pneumonia (Legionnaire’s disease): mandatory reporting

• Europe in 2013
  - 6012 cases reported by 29 countries with 11,4 notifications per millions inhabitants
  - 0.5 to 5% community-acquired pneumonia
  - 1st cause travel-associated pneumonia: 787 reported cases
  - <1% nosocomial pneumonieae (outbreak)

Reported cases of Legionnaires’ disease by month, UE, 2008-13

Reported cases of Legionnaire’s Disease per million, by reporting country, EU/EEA, 2013

Belgium 151 cases with a notification rate of 13.5/million inhabitants

Legionnaires’ disease: overview of the situation concerning notification in Wallonia (Belgium) in 2012, a retrospective descriptive study based on a capture-recapture method

Stéphanie Jacquinet¹, Olivier Denis²,³, Filomena Valente Soares³ and Carole Schirvel¹

Abstract

Background: Legionnaires’ disease is a severe form of pneumonia, and although public health medical inspectors must be notified, it is often under-reported. The objectives of this study were to determine the completeness rate of notification of Legionnaires’ disease and to estimate the incidence of this disease in Wallonia, the southern part of Belgium, in 2012.

Method: This retrospective, transversal descriptive study was based on a capture-recapture method using two sources. An estimation of the total number of Legionnaires’ disease cases was calculated using Chapman and Seber’s estimators for small numbers, thereby allowing us to estimate the real incidence of this disease in Wallonia as well as the completeness rate of notification.

Conclusions: The notification rate of Legionella must be improved in Wallonia. Doctors should be made aware of the importance of diagnosing and reporting Legionnaires’ disease.

Keywords: Legionnaires’ disease, Surveillance, Belgium
Travel-associated cases of Legionnaires’ disease per destination country, EU/EEA, 2013, n=835

Mode of transmission

- The source is always the environment
- Inhalation of Legionella-containing aerosols generated by
  - Man-made structures such as fountains, building water systems, cooling towers, ...
  - Infection is caused if *Legionella* can reach the lungs, or, rarely, other organs
  - *Legionella* infect most often previously unhealthy, but also healthy persons.
- Person-to-person transmission never been reported unlike other pathogens including *Mycoplasma* and *Chlamydophila*
Aerosol

* An aerosol is not a spray although it can be formed from a spray by small droplets drying to leave suspended droplet nuclei
* Aerosols are formed by bubbles released at a water surface (concentration effect)
* An aerosol is not visible
* Small particles <5μm can remain in suspension in air for prolonged periods and can be inhaled deep into the lungs
* Aerosols can travel long distances
Aerosol formation

• Water drops falling onto a hard surface
• Bubbles rising to the water surface and bursting
• Rain
• Running a tap
• Running shower
• Flushing a toilet
• Spraying plants
• Humidifiers
• Water running over pack of cooling towers
• Wave formation
Distribution of sampling sites testing positive for Legionella, EU, 2013

# Transmission from cooling towers

<table>
<thead>
<tr>
<th>Distance</th>
<th>Outbreak</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>150 m</td>
<td>85 cases medical centre Vermont</td>
<td>Am J Epidemiol 1984</td>
</tr>
<tr>
<td>30 m</td>
<td>15 cases at hospital Rhode Island</td>
<td>JAMA 1985</td>
</tr>
<tr>
<td>400 m</td>
<td>Delaware community outbreak caused by hospital cooling tower</td>
<td>Inter J Epidem 1999</td>
</tr>
<tr>
<td>1,7 km</td>
<td>33 cases in Glasgow</td>
<td>Lancet 1986</td>
</tr>
<tr>
<td>3,2 km</td>
<td>29 cases in Wisconsin</td>
<td>Am J Epidemiol 1989</td>
</tr>
<tr>
<td>≥ 6 km</td>
<td>86 cases (18 death) Lens, France</td>
<td>J Inf Dis 2006</td>
</tr>
</tbody>
</table>

**Long distance**
Commune-specific attack rates and geographical distribution of selected cases of legionnaires disease, Pas-de-Calais, France, November 2003–January 2004

Nguyen et al JID 2006
Clinical manifestations
Pneumonia or Legionnaires’ Disease

- **Incubation**
  - 2 to 10 days

- **Clinical and radiographic presentations**
  - Indistinguishable from other cause of pneumonia
  - Mild to severe illness
  - With initially unilateral and patchy infiltrates evolving to bilateral consolidation
  - Small pleural effusion (rare)
  - Other: T°; abdominal pain, nausea, vomiting and diarrhea; neurological symptoms; hyponatremia; hepatic dysfunction

- **High case-fatality rate**
  - Up to 30%, higher than other cause of CAP
  - Evolution dependent of host factors and therapy
Other clinical manifestations

- **Pontiac fever**
  - Incubation: 1 to 2 days
  - Self-limited, short-duration febrile illness,
  - Fever, chills, headache, myalgia's, malaise, for less than one week
  - Usually diagnosed during an outbreak

- **Extra-pulmonary infections**
  - Rare always in immunocompromised patients
  - Often occur as metastatic complications of pneumonia
  - Miscellaneous findings: abscesses and other infections of the brain, spleen or lymph nodes, and skeletal and myocardial muscles, ... prosthetic valve endocarditis

- **Asymptomatic**
  - Frequent
  - Diagnosed in outbreak setting
Mercante JW et al. CMR 2015

1. Legionella spp. in natural fresh water
2. Distribution
3. Colonization
4. Amplification
5. Aerosolization
6. Human exposure
7a. Legionnaires' Disease
7b. Pontiac Fever
7c. No infection/Asymptomatic

Legend:
- Established
- Established (Water)
- Established (Soil)
- Not established (Soil)

More susceptible/high-risk host
Less susceptible/low-risk host
Risks factors

**Individual**

- Immunocompromised patients* (organ transplantation, chemotherapy, radiotherapy, corticosteroid; uncommon in HIV infection)
- Elderly*
- Male > female
- Dialysis patients
- Host with chronic disease (heart, lung, kidney, diabetes)
- Alcoholics and smokers (COPD!)
- Patients undergoing major surgery
- Neonates with VAP

* = higher risk to develop fatal infection

**Environmental**

- Travel (inside country or abroad)
- Stay at proximity of aerosol producing systems (whirlpools, cooling towers, industrial complexes)
Case definition

• Probable case
  - Clinical criterion + at least one laboratory criterion for a probable case

• Confirmed case
  - Clinical criterion + at least one laboratory criterion for a confirmed case

Clinical Criteria

• Any person with pneumonia

Laboratory criteria

• For case confirmation
  - Positive culture of *Legionella spp*
  - Positive urinary Ag for *L. pneumophila*
  - Significant rise in specific antibody level to *L. pneumophila* type 1 in paired serum

• For probable case
  - Positive PCR for Legionella spp
  - DFA staining with monoclonal antibody
  - Significant rise in specific antibody level to other Legionella spp
  - Single high level of specific antibody to *L. pneumophila*
Diagnostic laboratory methods, 2005-2012, EU

Proportion of cases reported with culture, PCR and single high titre, 2008-13, EU

Culture methods

Method
- Samples from lower respiratory tract
- Special media is needed (BCYE with and without antibiotics)

Performance
- Sensitivity High >60% and 100% specific
- All *L. pneumophila* serogroups can be detected
- The method is confirmatory
- Isolation of the causative agent for genotyping in case of outbreak

Limitations
- Culture depending on the quality of the material obtained from the patient, quality of media, procedure and lab experience
- Other *Legionella spp* appear as uncharacteristic colonies after several days
- Growth is generally slow (3 to 7 days)
Proportion of cases diagnosed by culture: selected countries, EU

Identification of *Legionella spp*

**Standard phenotypic methods**
- Morphology of the colonies
- Grows on Blood agar (or without cystein)
- Autofluorescence?
- Latex agglutination test
  - *L. pneumophila* (Lp1/Lp 2-15)
  - Further tests are required for other *Legionella spp*

**Maldi-Tof**
- Highly discriminant
- Good performance for species identification

**Molecular methods**
- Sequence analysis of *mip* gene
- Free database hosted by Public Health England (access still available via www.ewgli.org)
Urinary antigen test

**Methods**
- Urine by immunochromatographic assay or ELISA
- 15 minutes assay

**Performance**
- Good sensitivity (60-95%) and excellent specificity (>99%)
- Concentration of urine increases sensitivity
- Positive detection one day after onset of symptoms
- The method is confirmatory

**Limitation**
- Only detection of *L. pneumophila* serotype 1
- Not possible to (sub)type the causative agent for outbreak investigation
- Samples can be false positive (especially if not heat treated)
- Possible prolonged Ag excretion for several months especially in immunocompromised patients
Serology

**Methods**
- Serum, commercial assay can be automated

**Performance**
- High sensitivity (> 75%) if the right antigens are used and samples are collected timely
- All *L. pneumophila* serogroups (and species) can be detected
- Confirmatory if significant rise in antibody for *L. pneumophila* sg 1 demonstrated
- Outbreak investigation

**Limitation**
- Dependent of the patient’s antibody response, the time can vary considerably from patient to patient (few days to weeks) before positive levels can be detected
- Dependent on well timed samples: one acute phase and one convalescent phase sample
- High antibody level can persist for a long period (weeks, month and years)
- High proportion of “false” positive samples are seen especially for *L. pneumophila* non-serogroup 1 serogroups and species
- Asymptomatic and mild infection can also elicit a response
**Molecular methods**

**Methods**

- PCR on respiratory samples including sputum, BAL, ...
- Method fast in few hours (faster than culture)

**Performance**

- High sensitive (75-95%) and good specificity (>95%)
- All *L. pneumophila* serogroups (and species) can be detected
- The infection can be diagnosed from the first day of admission to hospital
- DNA typing (SBT) can be performed directly on the patient sample
- *Legionella* can be cultured directly from the PCR positive sample

**Limitations**

- Not confirmatory
- The method requires expensive laboratory equipment
- False positive reactions due to contamination
- Not all patients produce sputum in the acute phase
Conclusion

- Environmental origin
- *Legionella pneumophila* cause severe disease particularly in immunocompromised patients
- First cause of travel-associated pneumonia
- Difficult diagnosis
  - Requiring combination of methods
  - Including Ag detection, PCR and culture
- Mandatory reporting
  - Underreported and probably underdiagnosed
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